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Biomarkers-Turning Failures into Success

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Abstract- Biomarkers are very important indicators of normal and abnormal biological processes. Although the term biomarker is relatively new, biomarkers have been used in pre-clinical research and clinical diagnosis for a considerable time. For example, body temperature is a well-known biomarker for fever. Blood pressure is used to determine the risk of stroke. It is also widely known that cholesterol values are a biomarker and risk indicator for coronary and vascular disease, and that C-reactive protein (CRP) is a marker for inflammation. According to National Institutes of Health a biomarker is "a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes or pharmacologic responses to a therapeutic intervention". This definition captures the clinical applications of biomarkers, including population screening, diagnosis, prognosis, monitoring, and prediction of therapeutic response or toxicity. Biomarkers may be used alone or in combination to assess the health or disease state of an individual. Also the discovery and validation of biomarkers is a time consuming and challenging process. In this paper a variety of issues are addressed including the sundry definitions, requirements, types, classifications and phases of evaluation of biomarkers. Furthermore, an overall overview is presented that how biomarkers were turning failures into success.

Keywords: Biomarkers, therapeutic, validation.

1. INTRODUCTION:

In 2001, a consensus panel at the National Institutes of Health defined the term biomarker as 'a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention or other health care intervention'. The biomarker is either produced by the diseased organ (e.g., tumor) or by the body in response to disease. Biomarkers are potentially useful along the whole spectrum of the disease process. Before diagnosis, markers could be used for screening and risk assessment. During diagnosis, markers can determine staging, grading, and selection of initial therapy. Later, they can be used to monitor therapy, select additional therapy, or monitor recurrent diseases [Atkinson A J *et al,* (2001*)*] Thus, identifying biomarkers include all diagnostic tests, imaging technologies, and any other objective measures of a person's health status. Biomarkers can also be used to reduce the time factor and cost for phase I and II of clinical trials by replacing clinical endpoints. Biomarkers span a broad sector of human health care and have been around since the understanding of human biology and diseases began to evolve. So, why is so much attention being paid to biomarkers today? Genetics, genomics, proteomics, and modern imaging techniques and other high throughput technologies allow us to measure more markers than before. In addition, we achieve a greater understanding of disease pathways, the targets of interventions, and the pharmacologic consequences of medicines.

Timely recognition of an ongoing pathological process is a crucial factor that influences a patient's chances for successful treatment ["The Biomarkers Consortium" , Etzioni R and Urban N *et al*,(2003)]. To accelerate and facilitate the determination of diagnosis, current medicine strongly relies on the specialized assessment of certain molecules, where the concentration of these molecules in a biological sample more or less correlates with the occurrence of a given disease. Determination of the concentration change of such biomarkers may allow screening of high-risk individuals and detect disease at early, still well curable stages, as well as facilitate the prognosis prediction and monitoring of treatment response. The ultimate goal of implementing these biomarkers in routine clinical tests is the reduction of morbidity and mortality. Unfortunately, even with these tools, it is not always easy to realize the full potential of well-established markers [,Andriole Gl *et al,(*2009) and Schroder Fh *et al,* (2009)].

2. AN IDEAL BIOMARKER

An ideal biomarker has certain characteristics that make it appropriate for checking a particular disease condition. Ideally, an ideal marker should have the following features:

- Safe and easy to measure
- Cost efficient to follow up
- Modifiable with treatment
- Consistent across gender and ethnic groups
- To correlate with disease burden, and thus provide information about disease activity.

3. BIOMARKER REQUIREMENTS

Biomarkers are becoming more and more important, especially when strong side effects are expected from the treatment of a chronic disease because they can confirm a difficult diagnosis or even make it possible in the first place[Thomson Reuters,(2008)].A number of diseases, such as Alzheimer's disease or rheumatoid arthritis, often begin with an early, symptom-free phase. In such symptom-free patients there may be more or less probability of actually developing symptoms. In these cases, biomarkers help to identify high-risk individuals reliably and in a timely manner so that they can either be treated before onset of the disease or as soon as possible thereafter [Craig-Schapiro R *et al*, (August 2009) and Egerer K *et al*, (March 2009)].

In order to use a biomarker for diagnostics, the sample material must be as easy to obtain as possible. This may be a blood sample taken by a doctor, a urine or saliva sample, or a drop of blood like those diabetes patients extract from their own fingertips for regular blood-sugar monitoring.

For rapid initiation of treatment, biomarker test should be critical. A rapid test, which delivers a result after only a few minutes, is optimal. This makes it possible for the physician to discuss with the patient how to proceed and if necessary to start treatment immediately after the test.

Naturally, the detection method for a biomarker must be accurate and as easy to carry out as possible. The results from different laboratories may not differ significantly from each other, and the biomarker must naturally have proven its effectiveness for the diagnosis, prognosis, and risk assessment of the affected diseases in independent studies.

A biomarker for clinical use needs good sensitivity and specificity e.g. ≥0.9, and good specificity e.g. ≥0.9 [Brower V (March 2011)] although they should be chosen with the population in mind so positive predictive value and negative predictive value are more relevant.

4. BIOMARKER CLASSIFICATION:

Biomarkers can be classified based on different parameters. They can be classified based on their characteristics such as:

(1) Imaging biomarkers (CT, PET, MRI)
(2) Molecular biomarkers can be used to refer to nonimaging biomarkers that have biophysical properties, which allow their measurements in biological samples (e.g., plasma, serum, cerebrospinal fluid, bronchoalveolar lavage, biopsy)
(3) Nucleic acids-based biomarkers such as gene mutations or polymorphisms and quantitative gene expression analysis, peptides, proteins, lipids metabolites, and other small molecules.
(4) Diagnostic biomarkers (i.e., cardiac troponin for the diagnosis of myocardial infarction), staging of disease biomarkers (i.e., brain natriuretic peptide for congestive heart failure), disease prognosis biomarkers (cancer biomarkers), and biomarkers for monitoring the clinical response to an intervention (HbAlc for antidiabetic treatment).
(5) Another category of biomarkers includes those used in decision making in early drug development. For instance, pharmacodynamic (PD) biomarkers are markers of a certain pharmacological response, which are of special interest in dose optimization studies.

4. TYPES:

A wide range of biomarkers are used today. Every biological system has its own specific biomarkers. Many of these biomarkers are relatively easy to measure and form part of routine medical examinations.Biomarkers validated by genetic and molecular biology methods can be classified into three types [Firestein, Gary (2006)].

- Type 0 — Natural history markers that correlate longitudinally with known clinical indices, such as symptoms over the full range of disease states.

- Type 1 — Drug activity markers capture the effects of an intervention in accordance with the mechanism of action of the drug, even though the mechanism might not be known to be associated with clinical out-come.
- Type 2 — Surrogate markers or surrogate end points because a change in that marker predicts clinical benefit. Unambiguous definitions were proposed to distinguish biomarkers from clinical endpoints, enabling debate on the validation and application of surrogate endpoints.

5. PHASES OF EVALUATION OF BIOMARKERS:

Because of diseased tissue/tumour heterogeneity and other biases that might be inherent with biomarker identification and evaluation processes, it is important that the identification of biomarkers should proceed in a systematic manner. Unlike a clinical trial design in which there are three phases (phase I, phase II and phase III), research on biomarkers has largely been guided by intuition and experience. In 2002, the National Cancer Institute's 'Early Detection Research Network' developed a five-phase approach to systematic discovery and evaluation of biomarkers. In general, biomarker development should follow an orderly process wherein one proceeds to the next phase only after meeting pre-specified criteria for the current phase [Sullivan Pepe M (2001)].

5.1 Phase 1:

It refers to preclinical exploratory studies. Biomarkers are discovered through knowledge based gene selection, gene expression profiling or protein profiling to distinguish cancer and normal samples. Identified markers are prioritized based on their diagnostic/prognostic/therapeutic (predictive) value that could suggest their evolution into routine clinical use. The analysis of this phase is usually characterized by ranking and selection, or finding suitable ways to combine biomarkers. Although not required, it is preferred that the specimen for this phase of discovery comes from well-characterized cohorts, tissue banks or from a trial with active follow-ups.

5.2 Phase II:

It has two important components. Upon successful completion of phase I requirements, an assay is established with a clear intended clinical use. The clinical assay could be a protein-, RNA-, DNA- or a cell-based technique, including ELISA, protein profiles from MS, phenotypic expression profiles, gene arrays, antibody arrays or quantitative PCR. To document clinical usefulness, firstly, such assays need to be validated for reproducibility and shown to be portable among different laboratories. Secondly, the assays should be evaluated for their clinical performance in terms of 'sensitivity' and 'specificity' with thresholds determined by the intended clinical use.

5.3 Phase III:

During this phase an investigator evaluates the sensitivity and specificity of the test for the detection of diseases that have yet to be detected clinically. The specimens analyzed in this evaluation phase are taken from study subjects before the onset of clinical symptoms, with active follow-up to ascertain disease occurrence. It is usually time consuming and expensive to collect these samples with high quality; therefore, phase III should consist of large cohort studies or intervention trials whenever possible. This is probably when most biomarker validation studies will end and the biomarker will be ready for clinical use.

5.4 Phase IV:

It evaluates the sensitivity and specificity of the test on a prospective cohort. The major difference from phase III is that in phase IV a positive test triggers a definitive diagnostic procedure, often invasive and that could lead to increased economic healthcare burden. Therefore, in a phase IV study, an investigator can estimate the false referral rate based on tested biomarkers and describe the extent and characteristics of the disease detected (e.g., the stage of tumors at the time of detection). For rare diseases, phase IV requires a large cohort with long-term follow-up and might often be too expensive as a stand-alone activity. These studies are difficult to perform specifically for rare diseases.

5.5 Phase V:

It evaluates the overall benefits and risks of the new diagnostic test on the screened population. The cost per life saved is one example of an endpoint for such a study. This again requires a large-scale study over a long time period and could also be prohibitively expensive.

Phases IV and V are necessary to evaluate benefits and risks of the use of a biomarker in screening and detection.

6. BIOMARKER USE ACROSS THE SPECTRUM OF DISEASES

6.1 Risk assessment

Risk assessment is the evaluation of the risk posed to human health by the actual or potential presence of specific risk characteristics both by qualitative and quantitatively. For example, cardiovascular risk assessment by tables and charts based on the Framingham equation are widely used [Wilson P W *et al,(*1998)]. Various biomarkers have been used to improve prediction by Framingham score. Lipoprotein-associated phospholipase A2, vitamin B6, IL-6, C-reactive protein and soluble thrombomodulin have been used [Folsom A R, *et al(* 2006)].

6.2 Screening

In Screening particular groups were selected which discriminates the healthy from the asymptomatic disease state. Biomarkers are important for screening and also in early diagnosis. For example, the prognosis of advanced HCC is poor, whereas smaller HCC suitable for organ transplantation, surgical resection or radio frequency ablation have shown a better prognosis and longer survival. Therefore, detection of HCC at an early stage heavily affects the clinical outcome of these patients. For this reason, a surveillance program using alpha fetoprotein (AFP) and ultrasound (US) every six months has been recommended, and is widely practised. So far, AFP, the only serological marker commonly used in diagnosis has failed to be a reliable marker mainly because it shows poor sensitivity, ranging from 39% to 65% and a specificity ranging from 76% to 97%. AFP seems to be reliable at values over 400 IU/ml, but the percentage of patients with such high levels is very small; this represents one of the most important limits of this marker. Various other markers for HCC diagnosis have been evaluated including fucosylated variant of the AFP glycoprotein, having a high affinity of the sugar chain to *Lens culinaris* (AFP-L3), hepatoma-specific AFP and AFP-mRNA, Des-gamma carboxy prothrombin (DCP), Glypican-3 (GPC3), squamous cell carcinoma antigen (SCCA), immunoglobulins of the IgM class forming complexes with either AFP (AFPIC) or SCCA (SCCAIC), tissue polypeptide specific antigen, hepatoma-specific gamma-glutamyl transferase isoenzyme, transforming growth factor (TGF)-β1 and TGF-β1- mRNA, insulin-like growth factor (IGF)-II and IGF-II mRNA and genetic alterations of telomerase. However, individually used, these markers don't have good performance characteristics. The combination of SCCA, SCCAIC, AFP and AFPIC has been investigated. This combination of biomarkers allows the identification of almost 80% of tumours with normal AFP, that represent the most difficult challenge for clinicians [Giannelli G *et al*, (2007)].

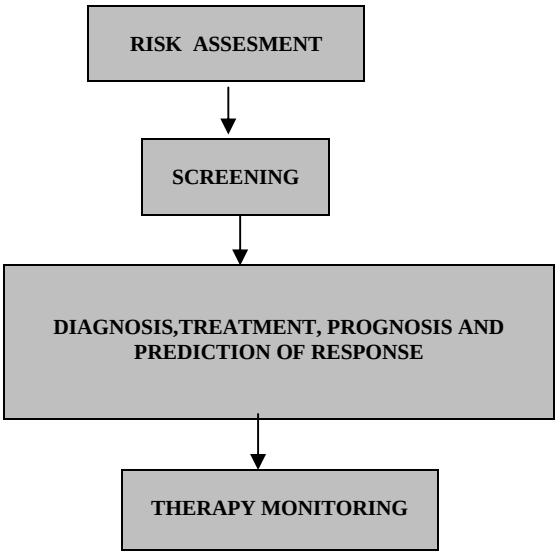

Figure 1. Schematic representation of the uses of biomarkers across the spectrum of diseases. Before diagnosis, markers might be used for risk assessment and screening. At diagnosis, markers can assist with staging, grading, and selection of initial therapy. Later, they can be used to monitor therapy, select additional therapy, or monitor for recurrent disease.

6.3 DIAGNOSIS, TREATMENT, PROGNOSIS AND PREDICTION OF RESPONSE

6.3.1 Classification, grading and staging

Classification of the tissue of origin of a disease especially malignancy is the first step towards predicting survival and choosing therapy. Because a

tumour's anatomical location usually indicates its tissue of origin, molecular markers are rarely required. Histological examination generally confirms the diagnosis and identifies the tumour subtype. However, in the differential diagnosis new molecular markers might sometimes be helpful. By using a combination of high-throughput RNA, protein and tissue microarray technologies, markers potentially useful for distinguishing colon and ovarian abdominal carcinomas from an unknown primary location can be identified [Nishizuka S *et al, (*2003)].

Each anatomical site has its own histological grading system, designed to classify malignancies by degree of differentiation. Low-grade, well differentiated tumours are usually less aggressive and more favourable in prognosis than high-grade tumours, which tend to grow faster and metastasize earlier. However, tumour grade is included in formal TNM staging only when intimately linked to prognosis, as it is for soft-tissue sarcomas, prostate cancer and primary brain malignancies. Assignment of grade is inherently subjective and dependent on the skill and experience of the reviewing pathologist, but several reports indicate that biomarker patterns can correctly score tumours according to their pathologist-assigned grades. Computer-aided diagnostic systems (CAD systems) have been used for preliminary grading of cervical smears and for assisted interpretation of radiological images such as screening mammograms, computerized tomography (CT) scans and standard X-ray films [Erickson B J and Bartholmai B (2002)]. CADs are generally designed to make routine distinctions, giving the pathologist time to focus on difficult diagnostic problems. The TNM Committee of the International Union Against Cancer (UICC), has defined staging criteria for most anatomical sites. T, N and M are determined separately and then grouped, usually to classify the cancer into one of four main stages (stages I–IV) and subdivisions thereof. Clinical staging , which is primarily used to guide initial therapy integrates information from physical examination with data such as those from standard X-ray, CT, MRI, PET, endoscopic examination, biopsy, and surgical exploration. Pathological staging on the basis of surgical specimens, if acquired, complements clinical staging with a precise determination of the extent of disease and additional histological information. Increasingly, imaging agents targeted at biomarkers are being used for anatomical localization. The most common are radioisotopes, detected by standard nuclear medicine imaging, by single-photon emission computed tomography (SPECT) or by PET. Also under study are fluorescent molecules, which are detected by optical imaging, and paramagnetic particles for enhancing MRI. The target can be any marker that delineates the cancer or its metabolism. For example, (18)F-FDG, (11)C-acetate, and dual-tracer PET/CT have recently been shown to have a relatively high sensitivity for the detection of extrahepatic metastases of HCC and may be potentially helpful in HCC staging [Park J W *et al*,(2008)]. Some tumours (for example, carcinoid, phaeochromocytoma, and cancers of the prostate, thyroid and colon) can be targeted by specific radiolabelled ligands. Carcinoid tumours, for example, are often localized using a radiolabelled analogue of octreotide (111-indium pentetreotide), which avidly binds to the somatostatin receptor, a protein commonly overexpressed in those tumours. Nuclear medicine-based imaging modalities are also clinically useful for evaluating tumour-related phenomena including angiogenesis, apoptosis, proliferation, metabolism, hypoxia and drug resistance (such as P-glycoprotein function). Molecularly targeted functional imaging has enormous potential for staging, as it does for other aspects of diagnosis and management [Ludwig J A and Weinstein J N(2005)]. Staging could also be useful in non-malignant diseases. For example, from a clinical management viewpoint, accurately assessing the extent and progression of liver fibrosis in cases of chronic liver disease is important. Liver biopsy is the current gold standard but is poorly suited for active monitoring because of its expense and morbidity. Thus, development of alternatives that are safe, inexpensive, and reliable is a priority. There have been tremendous advances in biomarkers for non-invasive assessment and staging of liver fibrosis.

Table 1 shows the various blood biomarkers evaluated for staging of liver fibrosis. Routine laboratory tests [aspartate aminotransferase (AST) to alanine aminotransferase (ALT) ratio; gamma glutamyl transferase (GGT); cholesterol; platelet count; AST to platelet ratio and insulin resistance], various proprietary test panels ['PGA index,' which combines prothrombin time, GGT, and apolipoprotein A1, which was later modified to include alpha-2-macroglobulin ('PGAA index'); 'Fibrotest,' which combines α-2-macroglobulin, haptoglobin, GGT, apolipoprotein A1, and total bilirubin;], specialized tests of liver function [indocyanine green; sorbitol; galactose clearance tests; 13C-galactose breath test; 13C-aminopyrine breath test and MEGX test], serum ECM markers of fibrosis ['Fibrospect panel comprising hyaluronic acid, TIMP1, and −2-macroglobulin; collagen IV; collagen VI; amino terminal propeptide of type III collagen (PIIINP); apolipoprotein A-IV; complement C-4; serum retinol binding proteins; serum N-glycans etc.] have been assessed and are being developed for staging liver fibrosis [Rockey D C and Bissell D M (2006)].

Table 1. Blood markers used to detect and stage liver fibrosis.

NAME	COMPONENTS	Sensitivity/ Specificity for advanced fibrosis	PPV/NPV for advanced fibrosis
AST/ALT ratio	AST/ALT	53%/100%	100%/81%
'Forns' test	platelets, GGT, cholesterol	94%/51%	40%/96%
APRI	AST, platelets	41%/95%	88%/64%
PGA index	platelets, GGT, apolipoprotein A	91%/81%	85%/89%
Fibrotest	GGT, haptoglobin bilirubin, apolipoprotein A, alpha-2-macroglobulin	87%/59%	63%/85%
Fibrospect	hyaluronic acid, TIMP-1, alpha-2-macroglobulin	83%/66%	72%/78%
FPI	AST, cholesterol, HOMA-IR	85%/48%	70%/69%
ELF	collagen IV, collagen VI, amino terminal propeptide of type III collagen (PIIINP), matrix metalloproteinase 2 (MMP-2), matrix metalloproteinase 9 (MMP-9), tissue inhibitor of matrix metalloproteinase 1 (TIMP-1), tenascin, laminin, and hyaluronic acid (HA).	90%/41%	35%/92%

Abbreviations: AST, aspartate aminotransferase; GGT, gamma glutamyl transpeptidase; APRI, AST to platelet ratio index; TIMP-1, tissue inhibitor of metalloproteinase 1; ECM, extracellular matrix; HOMA-IR – homeostasis model assessment (for insulin resistance).

6.3.2 Prognosis and treatment selection

Tumour classification, stage and sometimes grade are used to assess prognosis. Biomarker expression often supplants or complements tumour classification, stage and grade when biologically targeted therapeutics are under consideration. Prominent examples include CD20 positivity for treatment of lymphomas with rituximab, HER2/NEU positivity for treatment of breast cancer with trastuzumab, BCR-ABL translocation for treatment of chronic myelogenous leukaemia (CML) with imatinib, and KIT or platelet-derived growth factor receptor-α (PDGFRA) positivity for treatmentof gastrointestinal stromal tumours (GIST) with imatinib [Rockey D C and Bissell D M (2006)]. Both prognosis and prediction of response are necessary for the selection of neoadjuvant or adjuvant chemotherapy. Tissue classification, TNM staging, molecular biomarkers, grade and other factors might be used in combination for that purpose. The combinations of variables might not be easy to analyse manually, but computer decision support systems (DSS) can make the assessments automatically [Ravdin P M *et al (*2001)]. Biomarkers can also be used to avoid idiosyncratic drug toxicity such as the sustained, life-threatening leukocyte suppression seen when mercaptopurine is given to leukaemia patients with homozygous mutations of the thiopurine methyltransferase (*TPMT*) gene [Relling M and Dervieux T (2001)].

6.4 THERAPY MONITORING

With advances in understanding of tumour biology, interest in molecular biomarkers of carcinogenesis has grown, both in terms of their prognostic significance and also their potential as therapeutic targets. For example, surgery, including transplantation, remains the only potentially curative modality for HCC, yet recurrence rates are high and long-term survival poor. The ability to predict individual recurrence risk and subsequently prognosis would help guide surgical and chemotherapeutic treatment. As understanding of hepatocarcinogenesis has increased, the myriad of genetic and molecular events that drive the hepatocarcinogenic disease process, including angiogenesis, invasion and metastasis, have been identified. A number of molecular biomarkers with prognostic significance have been identified in hepatocellular carcinoma (table 2) [Mann C D *et al*, (2007)].

Table 2. Molecular markers of prognostic significance in hepatocellular carcinoma.

Hepatocarcinogenic process	Potential prognostic marker

Proliferation, self-sufficiency in growth signals, insensitivity to antigrowth signals	p53*, nm-23, Rb, PTEN*, c-met*, C-myc*, cyclin A, cyclin D, cyclin E, p15, p16, p18, p19, p21, p27, p57, TGF-b, EGFR family, growth factors proliferation indices*
Avoidance of apoptosis	p53*, Bcl-2, Bcl-xL, Bax, Bak, Bcl-xS, survivin
Limitless replicative potential	Telomerase (including TERT)*
Sustained angiogenesis	MVD, VEGF*, HIF-1a*, NOS, bFGF, PD-EGF,tissue factor,endostatin/collagen XVIII, interleukin- 8, angiopoietin
Tissue invasion and metastasis	MMPs*, uPA, cadherin/catenin complex
Genomic instability	Chromosomal instability, aneuploidy*, microsatellite instability

Abbreviations: nm-23, non-metastatic protein-23; Rb, retinoblastoma gene; PTEN, phosphatase and tensin homolog; TGF-b, transforming growth factor beta; EGFR family, epidermal growth factor receptor family; TGF-a, transforming growth factor alpha; HB-EGF, heparin-binding epidermal growth factor; TERT, telomerase reverse transcriptase; MVD, microvessel density; VEGF, vascular endothelial growth factor; HIF-1a, hypoxia-inducible factor-1 alpha; NOS, nitric oxide synthase; bFGF, basic fibroblast growth factor; PD-EGF, platelet-derived endothelial growth factor; MMP, matrix metalloproteases; uPA, urokinase plasminogen activator.

Research into the molecular biology of hepatocarcinogenesis has identified a multitude of molecular biomarkers with potential prognostic significance. Markers of particular interest include p53-mutation, PTEN, c-met, c-myc, p18, p27, p57, serum VEGF, HIF-1a, MMP-2, -7, and -12, as well as proliferation indices, telomerase activity and aneuploidy. Combining panels of molecular biomarkers with more traditional histopathological characteristics may enable more accurate prediction of those at high risk of disease progression and more appropriate targeting of resources. In addition to biomarker expression in resected specimens or biopsy samples, further emphasis should be placed on the role of circulating serum biomarkers. Assessment of molecular biomarkers in serum (for example pre-operative serum VEGF), as well as other body fluids including urine, may allow formulation of pre-operative prognostic criteria to identify patients most likely to benefit from particular therapies, such as hepatic resection and transplantation, as well as predict those most likely to respond to different chemotherapeutic agents. It may be that high-risk patients achieve no advantage in undergoing hepatic resection compared to a less invasive treatment modality, such as tumour ablation, with its reduced morbidity, mortality, and cost. In addition ,the ability to stratify patients' prognoses pre-operatively would improve provision of patient information when obtaining informed consent, allow assessment of the need for adjuvant therapies, and facilitate comparative studies and clinical trials. Serum and urinary biomarkers may also have a potential role in screening for recurrent disease following treatment. Ho and colleagues [Ho M C, Lin J J, Chen C N *et al (*2006)] used microarray to identify 14 genes that could discriminate between those patients with vascular invasion from those without. They subsequently tested the prognostic value of this finding on a separate group, finding a significantly poorer disease-free survival in those patients predicted to have vascular invasion, and therefore to be at higher risk of recurrence. Work by Iizuka and colleagues based on microarray analysis identified a group of genes that could predict intrahepatic recurrence with a positive predictive value of 88% and a negative predictive value of 95% [Iizuka N and Oka M *et al,(* 2003).

However, many initially promising biomarkers have not been validated for clinical use. The premise behind the use of biomarkers in medicine is that an observation or measurement can be used as a proxy of a biological process and as an indicator that a specific disease is present. Problems can develop at many stages of biomarker discovery and validation that contribute to the short life span of many "newly discovered" biomarkers. Careful validation in independent datasets by independent in-vestigators and publication of the findings are probably the best way to identify a good biomarker. Biomarkers are especially valuable, as they can help to prioritise drug discovery resources by enabling early proof-of-concept studies for novel therapeutic targets. This is especially important for therapeutic indications in which assump-tions regarding the relevance of animal models to clinical disease are tested only in large late-phase, long-term clinical trials that can require extensive dose ranging.Biomarkers can often be developed using animals in vivo before transferring the methodology to the clinic, although some technologies, such as functional brain imaging, can be compromised by constraints of experimental protocols. Biomarkers are especially valuable for providing early tests of key programme hypotheses, particularly if changes can be measured in normal volunteer subjects during initial clinical trials. The

choice of biomarkers should always factor in the feasibility and ease of clinical use in the specific setting in which the biomarker will be deployed. It is important to anticipate that use by planning, in advance, rigorous validation in appropriate preclinical and clinical study designs. The time required to achieve this can be sub-stantial, and therefore biomarker development should begin simultaneously, and proceed in parallel with, the search for new therapeutics. Biomarkers that monitor specific physiological or pharmacological mechanisms can be used to select between multiple therapeutic targets for a drug by identifying those that are most sensitive to the inter-vention. Biomarkers can also reveal drug targets as well as optimize selection of molecules that interact with these targets for further development. Under the auspices of the Office of the Director, National Institutes of Health, the 'Biomarkers and Surrogate Endpoint Working Group' agreed on classification system for biomarkers 2.

The discovery and validation of biomarkers is a timeconsuming and challenging process, the difficulties of which are often underestimated. Errors and biases occur at all phases of the discovery and validation studies and include preanalytical factors (population selection, sample collection, processing and storage), analytical factors (aspects of the assay such as its analytical sensitivity, specificity and robustness) and post-analytical factors (such as data overfitting and interpretation) [Diamandis EP *et al*, (2009]. These biases and errors complicate the process of biomarker discovery and validation, and failure to identify and correct any one of these errors can lead to the "false discovery" of biomarkers. Barriers that preclude biomarkers to be brought into the clinic have been addressed in detail in recent reviews [Kulasingam V ,Diamandis EP(2008) and Diamandis EP (2010) and Pavlou MP and Diamandis EP *et al*]. Common biases and errors would be avoided if stringent guidelines and methodologies are followed such as the prospective-specimen-collection, retrospectiveblinded- evaluation (PRoBE) design [Pepe MS *et al*,(2008)] and the Standards for Reporting of Diagnostic Accuracy (STARD) statement [Bossuyt PM *et al*,(2003]. Despite the large number of potential pitfalls present in biomarker discovery studies, there have been thorough and well planned studies performed which have identified seemingly useful biomarkers for pancreatic cancer.

7. CONCLUSION:

However, at present the future of biomarkers appears to be tinged with a mixture of excitement and uncertainty. In part that uncertainty is predicated upon the fact that numerous disciplines and practitioners contribute to the biomarker effort. In order to provide direction, clarity of goals and continued fortification of the biomarker foundation, more organisation needs to be brought to bear. Although many novel biomarkers are discovered, very few turn out to be clinically useful. The problems and difficulties faced in biomarker discovery and validation are abundant and should not be underestimated when designing strategies and experimental studies. Researchers should make sure to use clearly annotated clinical specimens, use appropriate control groups, include large numbers of samples and standardize sample handling in order to generate reliable data [29]. In the future, integration of biomarkers, identified using emerging high-throughput technologies, into medical practise will be necessary to achieve 'personalization' of treatment and disease prevention.

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